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Laser Tissue Welding of Dura Mater and Peripheral Nerves: A Scanning Electron Microscopy Study

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Background and Objective: **In order to elucidate the mechanism of tissue welding, scanning electron microscopy (SEM) was used to investigate the ultrastructural changes on the surface of dura mater and peripheral nerves after CO_2 laser welding.**
Study Design/Materials and Methods: **The dura mater and the epineurium of the nerves was welded with a CO_2 laser at 100 mW with pulses of 1.0 s (spot size 320 μm), both with and without additional use of a protein solder (egg white). The specimens were immediately examined using SEM.**
Results: **The laser tissue bonding mechanism is collagen-to-collagen attachment. After laser irradiation, the collagen fibrils are swollen, densely packed, and fused together. When a protein solder is used, the coagulated solder forms a solid bridge between the tissue edges, which is melted on and between the collagen fibrils.**
Conclusion: **Laser welds in dura mater and peripheral nerves are the result of collagen-to-collagen bonding. In solder-assisted laser welds, the tissue connection is made by an internal and an external matrix of coagulated solder.** © 1996 Wiley-Liss, Inc.

Key words: collagen, dura mater, CO_2 laser, peripheral nerve, tissue fusion

INTRODUCTION

Laser-assisted tissue welding offers several advantages over conventional suturing methods, such as less trauma to the tissue, less inflammatory and foreign body reaction, and a faster surgical procedure [1,2].

Various laser system have been successfully used for tissue fusion, ranging from the argon laser (λ = 488–514 nm) to the CO_2 laser (λ = 10,600 nm) [3–6]. However, the choice of the laser system and laser settings used for tissue fusion is rather based on empirical information, as the mechanism of tissue welding is not yet fully understood.

Various protein "solders" have been used to increase the bonding strength of the welded tissue, suggesting that the amount and availability of certain proteins play a role in the bonding mechanism [7–9]. Understanding the mechanism responsible for the fusion could lead to an appropriate selection of laser wavelength, power density, pulse duration, spot size, and type of solder for the particular tissue to be welded. In this way, a combination of maximal bonding strength and minimal thermal damage can be achieved.

The aim of this study was to elucidate the mechanism of CO_2 laser tissue welding of dura mater and peripheral nerves, with emphasis on the alterations in surface morphology which occur during laser welding. Furthermore, the role of a protein solder in tissue welding was investigated. Welding of the tissues was performed both with and without the use of a protein solder, and the specimens were examined immediately after the experiments using scanning electron microscopy.

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MATERIALS AND METHODS

Dura mater (n = 10) and tibial nerves (n = 10) of New Zealand White rabbits (weighing 1.9–2.5 kg) of both sexes were used for this study. All animals were used in other experiments, and the tissue specimens were collected just before the animals were sacrificed. Immediately after tissue harvest, the tissues were used for the experiments. Each dura section and nerve were transected with a scalpel in two sections of approximately the same size.

For all procedures a CO_2 milliwatt laser (Cooper LS 860, Cooper LaserSonics Inc., Santa Clara, CA) was used in conjunction with an operating microscope at 40-fold magnification (OpMi-1, Zeiss Inc., West Germany) and a joystick micromanipulator (Cooper LaserSonics LS-11). The laser was operated in a cw mode using an electrical shutter (T 132, Optilas Inc., The Netherlands) with a foot switch to control the pulse duration. A spot size of 320 μm was used.

The pieces of dura mater were welded together at 100 mW with five single-laser pulses of 1.0 s, both with and without addition of a solder. In the solder group, the dura was first welded with one pulse of laser energy, which provided initial bonding, and then one drop of solder (egg white) was applied (using a 25-gauge needle) to the repair site. With the solder covering the repair site, the area was again irradiated with an additional four pulses of laser energy to further bond the tissue (Fig. 1a,b). In the nerve welding group, the opposite nerve ends were closely approximated, and the epineurium of one of the nerve sections was pulled over the other nerve section and welded to the underlying epineurium (Fig. 1c). In the solder group, the epineurium was welded in a similar way as the dura mater (Fig. 1d). The laser settings that were used (100 mW, 1.0 s pulse duration) have previously been shown to produce the strongest bonding strength in vitro in laser-assisted nerve repair [8]. The control group consisted of non-irradiated dura mater and nerves.

After the welding procedure, the specimens were fixed in Karnovsky's fixative, dehydrated in a graded acetone series, and dried by the critical point drying process. The specimens were mounted on aluminum stubs with colloidal silver paint, sputtered with gold-palladium, and examined in a scanning electron microscope (ISI-SS40, Japan). Both the surface of the tissues as well as cross sections through the welded site were examined.

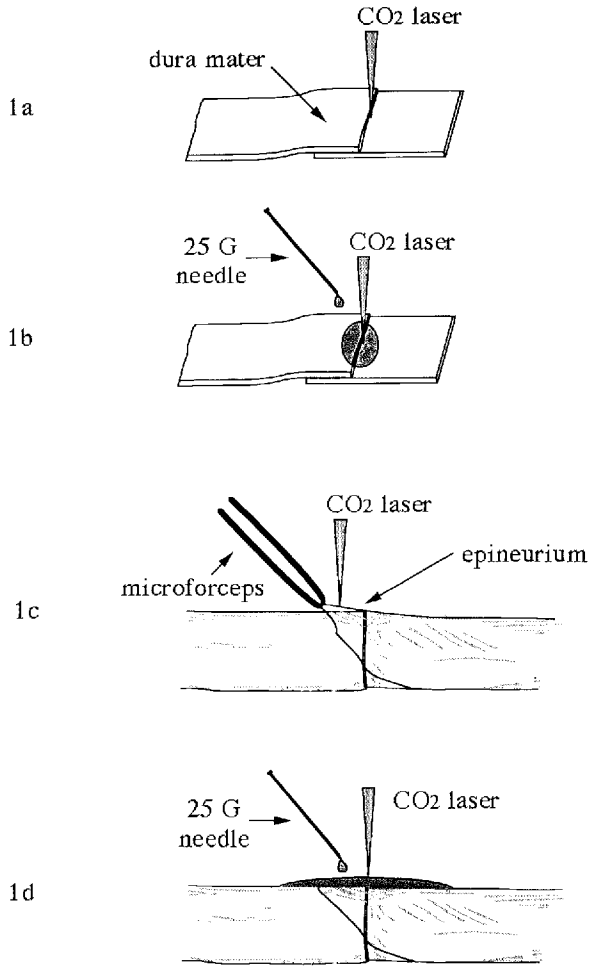


Fig. 1. **a–d:** The experimental procedure of CO_2 laser welding (see text).

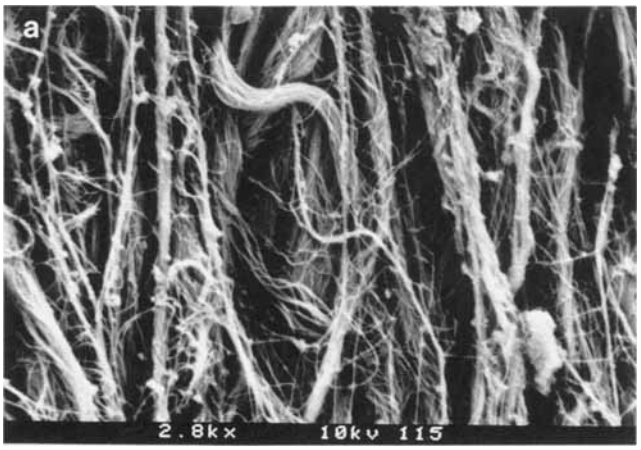
RESULTS

In all specimens there was evidence of adequate tissue bonding although it was observed during tissue manipulation that the bonding of the tissues was much stronger in the solder group. This observation was not quantified because SEM examination was the purpose of the study.

The appearance of the normal, non-irradiated dura mater and epineurium of the nerve is shown in Figure 2. Both tissues appeared as a thick fibrous mesh network consisting of mainly loose non-oriented interweaving strands of collagen fibrils.

Laser Welding

The dura mater and the epineurium of the nerve after welding had a specific appearance which was present in all specimens. From the outside, the repair site was easily identified as a



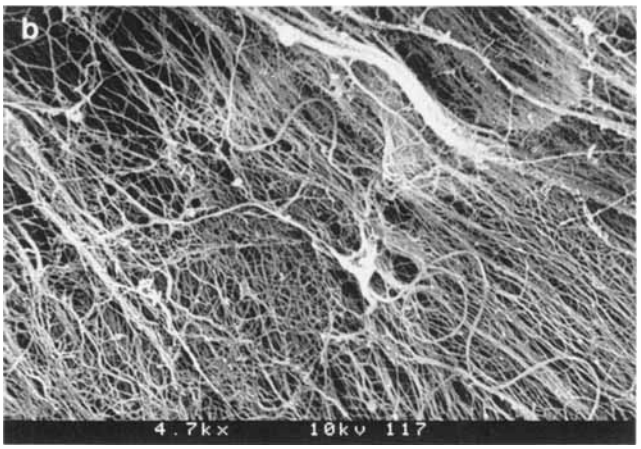


Fig. 2. Scanning electron micrograph of the normal dura mater (**a**) (×2,800) and the epineurium of a peripheral nerve (**b**) (×4,700).

slightly depressed area caused by the impact of the laser beam.

At the repair site, the fine loose structure of the collagen fibrils was lost. The individual fibrils were hardly recognizable and appeared to be homogeneously blurred and swollen (Fig. 3). The cutting edges of the tissue appeared to be merged by adherence and fusion of the collagen fibrils (Figs. 3, 4). Toward the periphery of the repair site, the individual fibrils were attached and tightly packed together (Fig. 5). The diameter of the fibrils was enlarged by an average factor of 6.5. The non-oriented interweaving strands of collagen fibrils had become aligned in a parallel fashion after laser irradiation (Fig. 5). At the border of the laser irradiation site, the occurrence of these changes decreased, and there was a transient shift to normal tissue. In several specimens, some coagulated tissue debris with a homogenous appearance adhering to the collagen fibrils could

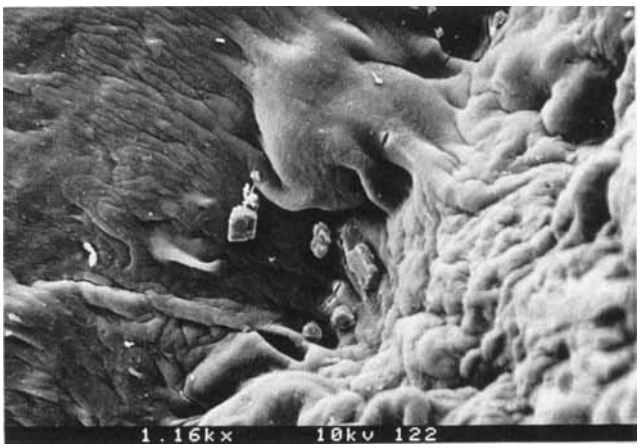


Fig. 3. Welded dura mater at the repair site. Note the homogenized and hardly recognizable collagen fibrils fused together (×1,160).

be observed, suggesting that this tissue debris acted as a microsolder. In general, there was no morphological difference between the welded dura mater and the epineurium.

Solder-Assisted Laser Welding

The solder became a solid glue after laser irradiation, covering the repair site. Therefore, the surface of the dura mater and peripheral nerve at the repair site was not visible. The coagulated solder appeared as a dense homogenous mass, elevated from the surface. At the direct repair site, the coagulated solder clung to the tissue, forming an outer stent (Fig. 6). On cross sections, the solder was intimately fused with the collagen fibrils, the contact between the solder and the tissue being very close (Fig. 6).

At the longitudinal border of the solder/tissue site, the solder had penetrated into the tissue and was fused on and between the collagen fibrils (Fig. 7). The collagen fibrils at the periphery of the repair site showed the same characteristic changes as in welding without the solder. In the cross sections, some small air bubbles could be observed within the coagulated mass of the solder (Fig. 8).

DISCUSSION

In the past, the mechanism of tissue welding has been studied with light and electron microscopy [2,4,10–13] and with biochemical analysis methods [14]. Especially transmission electron microscopy has enabled investigators to analyze

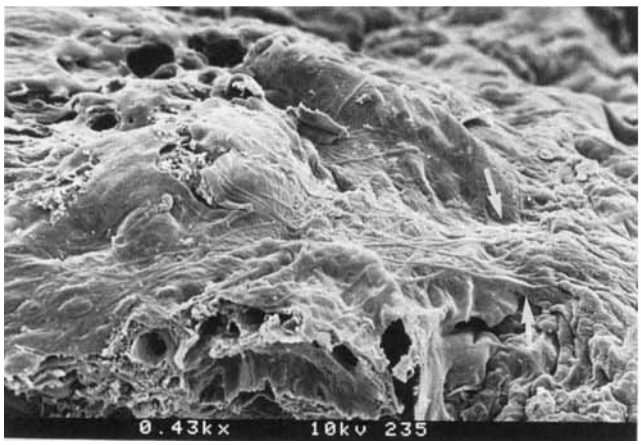


Fig. 4. Welded epineurium of the peripheral nerve. The arrows show the line of fusion between the two edges of tissue (×430).

Fig. 6. Longitudinal section through the welded peripheral nerve with the use of a solder. The white arrow shows the transection site, and the black small arrow shows the coagulated solder mass fused to the outer surface of the epineurium (×410).

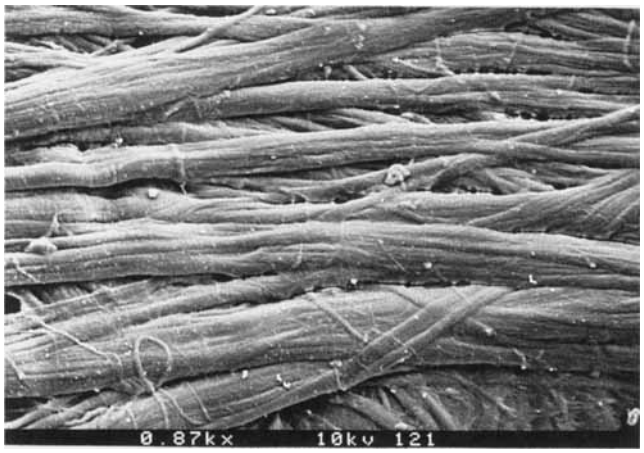


Fig. 5. Welded dura mater at the periphery. Note the packed collagen fibrils, are mainly parallel in orientation (×870).

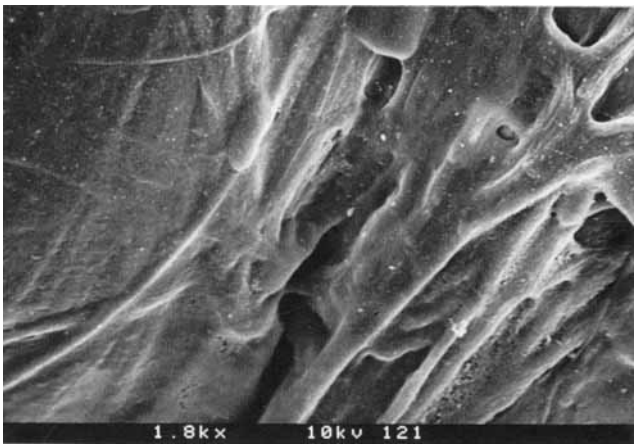


Fig. 7. The periphery of the repair site of a welded peripheral nerve with the use of a solder. The solder is fused on and between the collagen fibrils (×1,800).

the ultrastructural events associated with laser welding. However, few investigators used scanning electron microscopy (SEM) to investigate the three-dimensional changes of the tissue after CO_2 laser welding. We have used dura mater and epineurium of the peripheral nerves as a tissue model as these are mainly composed of collagen, which is believed to play a vital role in laser tissue welding [2]. Moreover, both tissues have been successfully welded with the CO_2 laser [15,16].

The mechanism of tissue welding is not yet fully understood as it is a process involving many variables. Furthermore, the alterations in tissue observed after laser irradiation are not necessarily responsible for the fusion. Although some authors have speculated that the mechanism may be in part wavelength dependent, it is considered to be primarily a thermal rather than a photochemical effect. The temperatures at which welding occurs have been reported to range between 60 and 120°C [11,17–21]. These temperatures are all above the denaturation temperature of collagen (60°C). Only Kopchok et al. [11] reported temperatures lower than 60°C during argon laser welding, and thus suggested a possible alternative mechanism (i.e., photochemical annealing of collagen fibrils).

Some proposed mechanisms of laser tissue welding include denaturation of structural proteins [22,23], dehydration of the proteins [24,25], acceleration of natural fibrinogen polymerization

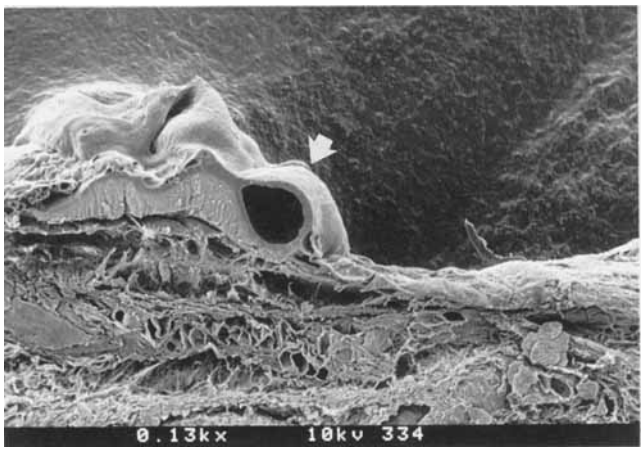


Fig. 8. Longitudinal cross section of a welded nerve with the use of a solder. The arrow shows a small air bubble (×130).

[26], collagen-to-collagen fusion [4,27], crosslinking of proteins [4], formation of non-covalent bonding between collagen [14,28], and interdigitation of collagen fibers [29]. The results of this study show that in laser welds the collagen undergoes specific changes like swelling and reorganization of the fibrils in one direction. This is in agreement with other studies using transmission electron microscopy [9,29,30]. Although the fusion area of the tissues consisted of collagen-to-collagen bonding, neither was the nature of these connections elucidated in this study, nor were the changes of collagen as seen on the SEM the proof that this forms the weld. These changes may be just the effect of laser heating. We did not find any interdigitation of the collagen fibrils as an underlying mechanism of tissue welding as reported by Schober et al. [29]. Because the laser welding procedure was performed in a bloodless field, neither erythrocytes nor fibrin could be attributed to the bonding process, as proposed by others [10,26, 31,32].

There seems no doubt that a solder contributes to the strength of the weld [7–9]. In this study, egg white was used as a solder, as egg white has been shown to produce stronger welds in vitro than other solders, such as albumin suspension, autologous fibrin glue, fibrinogen suspension, and red blood cells [8]. Egg white is composed mainly of water (88.1%), albumin (10.2%), and fat (0.05%) [33]. It is most likely that proteins are the main components responsible for the welding process. In repair without solder the bonding strength seems to originate from the collagen-to-collagen connections and possibly from cellular debris acting as a microsolder. In the soldered specimens, the coagulated solder acts as a dense homogeneous cast in which the collagen fibrils are embedded, acting both as an internal and an external glue. This explains the difference in the bonding strength of solder-assisted repair and repair without a solder, which was measured in a previous study [8].

Despite the encouraging results achieved with solders and egg white in particular, there are some technical, practical, and theoretical problems. First, the thickness of the solder layer applied to the repair side determines the outcome of the repair. A layer of solder which is too thick will result in a superficial coagulation of the solder, without coagulation and adhesion of the solder to the underlying tissue, producing a weak bond. This is especially the case with the CO_2 laser as the penetration depth of this laser in tissue is small (<100 μm), implying that the laser energy is absorbed mainly at the tissue surface. On the other hand, if the layer is too thin, the coagulated solder will not have the volume required to have any impact on the bonding process, resulting also in a relatively low bonding strength. Another important factor besides thickness is the energy fluence rate, which will have a substantial impact on the quality of the coagulation process. High energy fluence rates either will vaporize the solder or, especially in combination with a thick layer of the solder, will create bubbles (vaporized water inside the solder) which do not contribute to the weld strength and will weaken the repair site in the postoperative period. A low energy fluence rate will not sufficiently coagulate the solder. Therefore it is important to find an optimal fluence rate for a homogeneous solder coagulation. In this view, lasers with a slightly larger light penetration depth than the CO_2 laser might be better suited for solder-assisted tissue welding. Preliminary experiments using the Holmium:YAG milliwatt laser (λ = 2,09 μm, penetration depth = ~335 μm) revealed a perfect homogenous solder/tissue fusion in nerves (unpublished observation). However, further analysis is needed to quantify these observations. Another issue is the viscosity of the solder and the inherent penetration of the solder into the tissue. The more viscous the solder is, the less will be the penetration into the tissue, thus losing its role as an internal matrix upon coagulation.

The practical and theoretical problems that may arise with the use of egg white as a solder in

vivo is that the solder can probably initiate an immunological reaction of the host. As egg white is not sterile, the potential danger of viral or bacterial transmission is present. Another source of complications with the use of solders for nerve repair could be the persistence of solder between the tissue ends. If this occurs, the solder could block the sprouting axons and, as a result of a poor tissue-to-tissue apposition, secondary healing will follow with a higher incidence of scarring in nerves. This can also lead to aneurysm formation in welded vessels. Also, interference with healing and premature absorption and disintegration of the solder is possible, which may result in early dehiscention of the union. Finally, adhesion formation of the welded tissue to the surrounding tissue is possible. These are all factors which should be further investigated.

In view of our results and a review of the literature, we hypothesize that the most probable mechanism of CO_2 laser welding is thermal coagulation of tissue. As a result of the laser irradiation, cell membranes are disrupted, and the proteins of the cell leak, forming a microsolder of proteins, which together with the denatured collagen results in tissue adhesion. The proteins undergo a thermal degradation of the bonds (disulfide and hydroxyl bonds) and form new molecular bonds upon cooling, in the same fashion as boiling an egg, resulting in an adhesion of the proteins. These changes also occur in acellular tissues such as collagen. These alterations of molecular configuration do not have the tensile strength of the original structure [34,35]. Depending on the precision of tissue apposition, several different welds can be distinguished. In primary welds, there is an excellent tissue-to-tissue apposition resulting in close collagen-to-collagen bonds [4,36,37], allowing primary wound healing. In secondary welds, the tissue apposition is poor, resulting in a bond that is mainly formed by red blood cells, fibrin plug, and cell debris [10,38,39]. It is this kind of bond that produces aneurysm formation in vessel welding. In both primary and secondary welds, there is thermal damage to the tissue. Tertiary welds are completed with the use of solders or other materials, in which the tissue is held together by a cast of coagulated solder with almost no thermal damage.

In conclusion, we have demonstrated that 1) laser irradiation causes collagen-to-collagen bonding with specific changes in the collagen structure and 2) laser irradiation with the addition of a protein solder causes coagulation of the solder which forms a solid mass and a matrix in which the tissue is embedded.

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REFERENCES

1. Almquist EE. Nerve repair by laser. Orthop Clin North Am 1988; 19:201–208.
2. Neblett CR, Morris JR, Thomsen S. Laser-assisted microsurgical anastomosis. Neurosurgery 1986; 19:914–934.
3. Samonte BR, Fried MP. Laser-assisted microvascular anastomosis using CO_2 and KTP/532 lasers. Lasers Surg Med 1991; 11:511–516.
4. White RA, Kopchok GE, Donayre CE, Peng SK, Fujitani RM, White GH, Uitto J. Mechanism of tissue fusion in argon laser-welded vein-artery anastomoses. Lasers Surg Med 1988; 8:83–89.
5. Bass LS, Treat MR, Dzakonski C, Trokel SL. Sutureless microvascular anastomosis using the THC:YAG laser: A preliminary report. Microsurgery 1989; 10:189–193.
6. Burger RA, Gerharz CD, Rothe H, Engelmann UH, Hohenfellner R. CO_2 and Nd:YAG laser systems in microsurgical venous anastomoses. Urol Res 1991; 19:253–257.
7. Cikrit DF, Dalsing MC, Weinstein TS, Palmer K, Lalka SG, Unthank JL. CO_2-welded venous anastomosis: Enhancement of weld strength with heterologous fibrin glue. Lasers Surg Med 1990; 10:584–590.
8. Menovsky T, Beek JF, van Gemert MJ. CO_2 laser nerve welding: Optimal laser parameters and the use of solders in vitro. Microsurgery 1994; 15:44–51.
9. Poppas DP, Rooke CT, Schlossberg SM. Optimal parameters for CO_2 laser reconstruction of urethral tissue using a protein solder. J Urol 1992; 148:220–224.
10. Flemming AF, Colles MJ, Guillianotti R, Brough MD, Bown SG. Laser assisted microvascular anastomosis of arteries and veins: Laser tissue welding. Br J Plast Surg 1988; 41:378–388.
11. Kopchok GE, White RA, White GH, Fujitani R, Vlasak J, Dykhovsky L, Grundfest WS. CO_2 and argon laser vascular welding: Acute histologic and thermodynamic comparison. Lasers Surg Med 1988; 8:584–588.
12. Kopchok G, White RA, Grundfest WS, Fujitani RM, Litvack F, Klein SR, White GH. Thermal studies of in-vivo vascular tissue fusion by argon laser. J Invest Surg 1988; 1:5–12.
13. Poppas DP, Schlossberg SM, Richmond IL, Gilbert DA, Devine CJ. Laser welding in urethral surgery: Improved results with a protein solder. J Urol 1988; 139:415–417.
14. Bass LS, Moazami N, Pocsidio J, Oz MC, LoGerfo P, Treat MR. Electrophoretic mobility patterns of collagen following laser welding. SPIE 1991; 1244:123–127.
15. Hadley MN, Martin NA, Spetzler RF, Sonntag VK, Johnson PC. Comparative transoral dural closure techniques: A canine model. Neurosurgery 1988; 22:392–397.
16. Fischer DW, Beggs JL, Kenshalo DLJ, Shetter AG. Com-

parative study of microepineurial anastomoses with the use of CO_2 laser and suture techniques in rat sciatic nerves: Part 1. Surgical technique, nerve action potentials, and morphological studies. Neurosurgery 1985; 17: 300–308.
17. Badeau AF, Lee CE, Morris JR, Thomsen S, Malk EG, Welch AJ. Temperature response during microvascular anastomosis using CO_2 laser. Lasers Surg Med 1986; 6:202.
18. Brooks SG, Ashley S, Fisher J, Davies GA, Griffiths J, Kester RC, Rees MR. Exogenous chromophores for the Argon and Nd:YAG lasers: A potential application to laser-tissue interactions. Lasers Surg Med 1992; 12:294–302.
19. Epstein M, Cooley CB. Electron microscopic study of laser dosimetry for microvascular tissue welding. Lasers Surg Med 1986; 6:202.
20. Martinot VL, Mordon SR, Mitchell VA, Pellerin PN, Brunetaud JM. Determination of efficient parameters for argon laser-assisted anastomoses in rats: Macroscopic, thermal, and histological evaluation. Lasers Surg Med 1994; 15:168–175.
21. Vance CA, Fisher J, Wheatley DJ, Evans JH, Spyt TJ, Moseley H, Paul JP. Laser assisted vessel anastomosis of coronary arteries in vitro: Optimization of bonding conditions. Lasers Med Sci 1988; 3:219–227.
22. Dew DK, Supik L, Darrow C2, Price GF. Tissue repair using lasers: A review. Orthopedics 1993; 16:581–587.
23. Okada M, Shimizu K, Ikuta H, Horii H, Nakamura K. A new method of vascular anastomosis by CO_2 laser: Experimental and clinical study. Nippon Geka Gakkai Zasshi 1987; 88:390–400.
24. Fenner J, Martin W, Moseley H, Wheatley DJ. Shear strength of tissue bonds as a function of bonding temperature: A proposed mechanism for laser-assisted tissue welding. Lasers Med Sci 1992; 7:39–43.
25. Fenner J, Moseley H, Martin W, Wheatley DJ. Strength of tissue bonds as a function of surface apposition. Lasers Med Sci 1992; 7:375–379.
26. Vale BH, Frenkel A, Trenka-Benthin S, Matlaga BF. Microsurgical anastomosis of rat carotid arteries with the CO_2 laser. Plast Reconstr Surg 1986; 77:759–566.
27. Godlewski G, Rouy S, Dauzat M. Ultrastructural study of arterial wall repair after Argon laser micro-anastomosis. Laser Surg Med 1987; 7:258–262.
28. Bass LS, Moazami N, Pocsidio J, Oz MC, LoGerfo P, Treat MR. Changes in type I collagen following laser welding. Lasers Surg Med 1992; 12:500–505.
29. Schober R, Ulrich F, Sander T, Durselen H, Hessel S. Laser-induced alteration of collagen substructure allows microsurgical tissue welding. Science 1986; 232:1421–1422.
30. DeCoste SD, Farinelli W, Flotte T, Anderson RR. Dye-enhanced laser welding for skin closure. Lasers Surg Med 1992; 12:25–32.
31. Vlasak JW, Kopchok GE, White RA. Laser-assisted intestinal anastomosis. Lasers Surg Med 1988; 8:573–578.
32. Reali UM, Gelli R, Giannoti V, Gori F, Pratesi R, Pini R. Experimental diode laser-assisted microvascular anastomosis. J Reconstr Microsurg 1993; 9:203–210.
33. Long C. "Biochemist's Handbook." London: E & F.N. Spon Ltd, 1961.
34. Flory PJ, Garett RR. Phase transitions in collagen and gelatin systems. J Am Chem Soc 1958; 80:4836–4845.
35. Gorish W, Boergen KP. Heat-induced contraction of blood vessels. Lasers Surg Med 1982; 2:1–13.
36. Fujitani RM, White RA, Kopchok GE, Peng SK, White GH, Klein SR. Biophysical mechanism of argon laser-assisted vascular anastomoses. Curr Surg 1988; 45:119–123.
37. Kuroyanagi Y, Taguchi M, Yano T, Jones DN, Shionoya S. Argon laser-assisted anastomoses in medium-size vessels: One-year follow-up. Lasers Surg Med 1991; 11:223–231.
38. Chow JWM, Flemming AFS. Laser assisted microvascular anastomoses: A histological study. Lasers Med Sci 1990; 5:281–287.
39. Unno N, Sakaguchi S, Koyano K. Microvascular anastomosis using a new diode laser system with a contact probe. Lasers Surg Med 1989; 9:160–168.